

CLAIMS

1. A glycosylation-deficient hepatocyte growth factor lacking the sugar chain(s) at all or at least one of the 5 glycosylation sites of hepatocyte growth factor.

2. The glycosylation-deficient hepatocyte growth factor according to claim 1, wherein a mutation is introduced into an amino acid sequence so that no 10 glycosylation occurs at at least one of the glycosylation sites of the hepatocyte growth factor.

3. The glycosylation-deficient hepatocyte growth factor according to claim 2, wherein at least one of the 15 following modifications of (a) to (d) are applied to the amino acid sequence of the hepatocyte growth factor:

(a) Asn in at least one of consensus sequences for N-glycosylation represented by Asn-X-Ser or Asn-X-Thr (X represents an amino acid except Pro), which exist in the 20 amino acid sequence of hepatocyte growth factor, is substituted by another amino acid residue;

(b) Ser or Thr in one consensus sequence, or Ser and/or Thr in two or more consensus sequences for N-glycosylation represented by Asn-X-Ser or Asn-X-Thr (X represents an amino acid except Pro), which exist in the 25 amino acid sequence of hepatocyte growth factor, is/are substituted by other amino acid residue(s),

(c) X in at least one of consensus sequences for N-glycosylation represented by Asn-X-Ser or Asn-X-Thr (X

represents an amino acid except Pro), which exist in the amino acid sequence of hepatocyte growth factor, is substituted by Pro, or

(d) at least one of Ser and/or Thr that undergoes O-glycosylation, which exist in the amino acid sequence of hepatocyte growth factor, is/are substituted by other amino acid residue(s).

4. The glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 3, wherein the hepatocyte growth factor is human hepatocyte growth factor.

5. The glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 3, wherein the hepatocyte growth factor is feline or canine hepatocyte growth factor.

6. The glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 4, which is modified based on the amino acid sequence of SEQ ID NO: 1, wherein at least one of modifications represented by (a) to (e) below is applied to the amino acid in SEQ ID NO: 1:

(a) substitution of amino acid 294 and/or 296 by another amino acid, and/or substitution of amino acid 295 by Pro, leading thereby to no glycosylation of the amino acid 294;

(b) substitution of amino acid 402 and/or 404 by another amino acid, and/or substitution of amino acid 403 by Pro, leading thereby to no glycosylation of the amino

acid 402;

(c) substitution of amino acid 476 by another amino acid, resulting in no glycosylation of the amino acid 476;

5 (d) substitution of amino acid 566 and/or 568 by another amino acid, and/or substitution of amino acid 567 by Pro, leading thereby to no glycosylation of the amino acid 566; or

10 (e) substitution of amino acid 653 and/or 655 by another amino acid, and/or substitution of amino acid 654 by Pro, leading thereby to no glycosylation of the amino acid 653.

7. The glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 4, which is modified based on the amino acid sequence of SEQ ID NO: 2, wherein at least one of modifications represented by (a) to (e) below is applied to the amino acid in SEQ ID NO: 2:

20 (a) substitution of amino acid 289 and/or 291 by another amino acid, and/or substitution of amino acid 290 by Pro, leading thereby to no glycosylation of the amino acid 289;

25 (b) substitution of amino acid 397 and/or 399 by another amino acid, and/or substitution of amino acid 398 by Pro, leading thereby to no glycosylation of the amino acid 397;

(c) substitution of amino acid 471 by another amino acid, leading thereby to no glycosylation of the amino acid 471;

(d) substitution of amino acid 561 and/or 563 by

another amino acid, and/or substitution of amino acid 562 by Pro, leading thereby to no glycosylation of the amino acid 561; or

5 (e) substitution of amino acid 648 and/or 650 by another amino acid, and/or substitution of amino acid 649 by Pro, leading thereby to no glycosylation of the amino acid 648;

10 8. A DNA comprising a base sequence encoding the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7.

15 9. A vector integrated with the DNA according to claim 8.

10 10. A method for producing the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7 comprising the steps of: introducing the vector according to claim 9 into a cell; culturing the cell; 20 producing a glycosylation-deficient hepatocyte growth factor in the cell or into the cell culture medium; and recovering and purifying the glycosylation-deficient hepatocyte growth factor from the cell or from the cell culture medium.

25 11. The method according to claim 10 for producing the glycosylation-deficient hepatocyte growth factor, wherein the cell is a eukaryotic cell.

12. The method according to claim 11 for producing the glycosylation-deficient hepatocyte growth factor, wherein the eukaryotic cell is a yeast or an insect cell.

5       13. A method for producing the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7, comprising the steps of: introducing the vector according to claim 9 into an insect individual, allowing the insect individual to produce the glycosylation-  
10 deficient hepatocyte growth factor, and recovering and purifying the glycosylation-deficient hepatocyte growth factor from the insect individual.

14. A method for producing the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7, comprising the steps of: removing the sugar chain(s) wholly or partially by treating hepatocyte growth factor having sugar chain(s) with an enzyme, and recovering and purifying the glycosylation-deficient hepatocyte growth  
20 factor from the enzyme reaction solution.

15. A method for producing the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7, comprising the steps of: introducing a vector integrated with a DNA containing a base sequence encoding hepatocyte growth factor having sugar chain(s) or the vector according to claim 9 into a cell having no glycosylation ability; culturing the cell; allowing the cell to produce a glycosylation-deficient hepatocyte growth

factor in the cell or into the cell culture medium; and recovering and purifying the glycosylation-deficient hepatocyte growth factor from the cell or cell culture medium.

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16. A method for producing the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7, comprising the steps of: synthesizing the glycosylation-deficient hepatocyte growth factor by a cell-free protein synthesis system using a gene comprising a base sequence encoding hepatocyte growth factor having sugar chain(s) or the base sequence according to claim 8 as a template and recovering and purifying the glycosylation-deficient hepatocyte growth factor from the reaction solution.

17. A pharmaceutical preparation comprising the glycosylation-deficient hepatocyte growth factor according to any one of claims 1 to 7 as an active ingredient.

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18. A gene therapy agent containing the DNA according to claim 8.